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Original article

Genetic differences between *Tropilaelaps clareae* and *Tropilaelaps koenigerum* in Thailand based on ITS and RAPD analyses

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Abstract – *Tropilaelaps koenigerum* was first reported in Thailand. Species-diagnostic markers of bee mites, *T. clareae* and *T. koenigerum* were examined by sequencing of ITS and RAPD analysis. A lack of intraspecific polymorphism within *T. clareae* and *T. koenigerum* ITS was observed. At an interspecific level, 19 substitutions were found. Sequence divergence between ITS of these bee mites was 3.82%. A 5 bp (TTCTC) insertion was found in *T. koenigerum.* Based on ITS sequences, identification of these mites was simplified to restriction analysis of the amplified ITS with *Mse* I and/or *Sau*3A I. RAPD analysis using primers OPA07, OPA11 and OPA12 revealed 16 and 20 species-specific markers of *T. clareae* and *T. koenigerum*, respectively. A UPGMA phenogram based on genetic distance between pairs of geographic samples indicated clear differentiation between *T. clareae* and *T. koenigerum* genetically. Partial differentiation of *T. clareae* from different host species was observed.

***Tropilaelaps* / bee parasite / RAPD / ITS / species-diagnostic marker / genetic diversity**

1. INTRODUCTION

Parasitic bee mites, *Tropilaelaps clareae* Delfinado and Baker and *T. koenigerum* Delfinado-Baker and Baker (Acari, Laelapidae) associate with the giant honeybee (*Apis dorsata* Fabricius and *A. laboriosa* F. Smith) naturally. *T. clareae* is now reported from the five honey bee species – *A. mellifera* L., *A. dorsata* Fabricius, *A. cerana* Fabricius, *A. florea* Fabricius and *A. laboriosa* F. Smith (Aggarwal, 1988). The distribution of *T. clareae* includes India, Pakistan, Philippines, Nepal and Burma whereas *T. koenigerum* has been reported from Sri Lanka, Nepal (Delfinado-Baker et al., 1985) Borneo (Koeniger et al., 2002) and first reported in Thailand from this study.

After the European honeybee (*A. mellifera*) was introduced to Asia and subsequently to Thailand, cross-species infection by *T. clareae* occurred resulting in a significant loss of commercial honey production annually (De Jong, 1990). Interestingly, *T. clareae* is more harmful to the exotic species, *A. mellifera* than the native host, *A. dorsata* (Eickwort, 1988).

At present, genetic relationships among *T. clareae* from different hosts are not understood. It is still unclear whether *Tropilaelaps* mites of *A. mellifera* are confined

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to a unified subgroup within *T. clareae* or include an array of clonal lineages. Recently, data concerning life cycle of female *T. clareae* on *A. mellifera* host were reported (Rath et al., 1994; Delfinado-Baker and Peng, 1995; Sammataro et al., 2000) but there have been no reports on that aspect in *T. koenigerum*.

Morphological studies of these closely related species were previously reported by Delfinado-Baker and Baker (1982) and De Jong (1990). Nevertheless, differentiation of *T. clareae* and *T. koenigerum* based principally on morphology is difficult and requires experienced taxonomists. Prior to the present study, there have been no reports on identification of *Tropilaelaps* species in Thailand using molecular genetic markers. Accordingly, genetic markers specifically found in each bee mite need to be developed.

The nuclear rRNA genes are organized in clusters of tandemly repeated units. Each of these consists of conserved regions (18S, 5.8S and 28S rDNAs) and more variable transcribed and nontranscribed regions: external transcribed spacer (ETS), internal transcribed spacers (ITS), and intergenic spacers (IGS), respectively (Beckingham, 1987; Hillis and Dixon, 1991).

Polymorphism of ITS is useful and has been widely used to differentiate closely related organisms, particularly at an interspecific level (Hillis and Davis, 1986; Hillis and Dixon, 1991; Odorico and Miller, 1997). However, several studies have utilized ITS sequences for intraspecific genetic analysis in invertebrates, for instance, determination of the North American tiger beetle, *Cicindela dorsalis* phylogeny (Vogler and DeSalle, 1994) and examination of concerted evolution and molecular drive in the black fly, *Simulium damnosum* (Tang et al., 1996).

Randomly amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) is a simple and rapid method for identification of useful genetic markers and determination of organismal genetic diversity at various taxonomic levels. The advantage of this technique is that no prior knowledge of the genome under study is needed (Welsh and McClelland, 1990; William et al., 1990; Weising et al., 1995).

The objective of this study was development of species diagnostic markers to assist taxonomic identification of *T. clareae* and *T. koenigerum* in Thailand. Specimens collected from different locations and host species were examined by DNA sequencing and RAPD analysis. Species identification was simplified from DNA sequencing to restriction analysis of the amplified ITS.

2. MATERIALS AND METHODS

2.1. Sampling

Ten colonies of *A. dorsata* and eight colonies of *A. mellifera* were collected from different geographic locations in Thailand (Fig. 1 and Tab. I). A colony of honey bees was treated as a sampling unit. Bee mites, *T. clareae* and *T. koenigerum* were isolated and identified morphologically on the basis of Delfinado-Baker and Baker (1982). Characterized specimens were kept under liquid nitrogen until further required.

2.2. DNA extraction

Total DNA was extracted from each specimen using a modification of the method of Walsh et al. (1994). Briefly, a frozen mite was homogenized in 35 μL of 5% Chelex. The homogenate was gently vortexed for 30 s, incubated at 55 °C for 3 h followed by 95–100 °C for 7 min. The sample was centrifuged at 8 000 *g* for 10 min at room temperature. The supernatant was collected and stored at 4 °C.

2.3. Sequencing of PCR-amplified ITS

ITS was amplified by PCR in a 25 μL reaction mixture containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 0.001% gelatin, 2 mM $MgCl_2$, 100 μM of each dNTP and 0.2 μM each of primers ITS5, 5'-GGAAGTAAAAGTCGTAACAAGG-3' and ITS4, 5'-TCCTCCGCTTATTGATATGC-3' (White et al., 1990), 1 unit of Ampli*Taq* DNA polymerase (Perkin-Elmer Cetus) and 4 μL of extracted DNA. The thermo-cycles were predenaturation at 94 °C for 1 min followed by 35 cycles of denaturation at 92 °C for 1 min, annealing at 52 °C for 1 min and extension at 72 °C for 2 min. The final extension was performed at 72 °C for 7 min. The 600 bp PCR product was electrophoretically analyzed and gel-eluted using a Geneclean II kit (Bio101). The eluted DNA was directly sequenced for both directions using an OmniBase™ DNA cycle sequencing system (Promega). ITS4, ITS5, inITS5 (5'-TCGTATGTATTCCATTCGTA-3') or

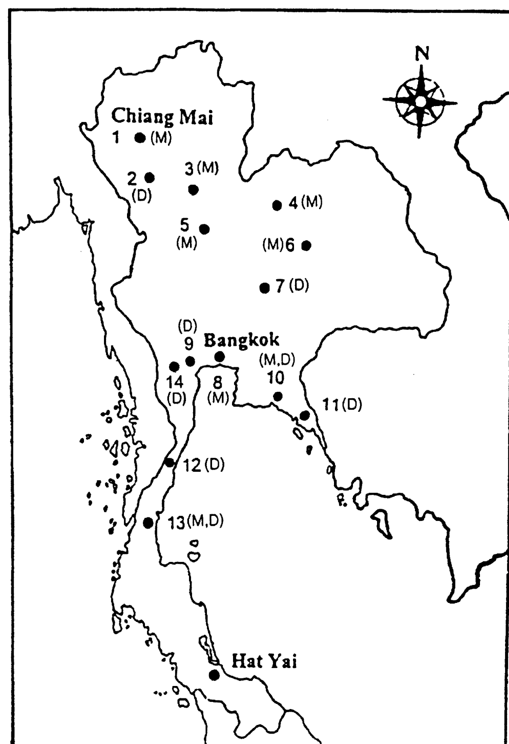

Figure 1. Map of Thailand showing sampling locations (1–14) of specimens used in this study. M and D represent host of bee mites, *A. mellifera* and *A. dorsata*, respectively.

inITS4 (5'-CATAGACACAAGGCATCCAT-3') were used as sequencing primers.

2.4. Restriction analysis of amplified ITS

Approximately 800 ng of PCR-amplified ITS of *T. clareae* (N = 30) and *T. koenigerum* (N = 10) were separately digested with 5 units of *Mse* I and *Sau*3A I (New England Biolabs) in a 25 µL reaction mixture at 37 °C for 3 h. The digests were analyzed by electrophoresis through 2.0% MetaPhor agarose gels (FMC) and visualized under a UV transilluminator after ethidium bromide staining (Maniatis et al., 1982).

2.5. RAPD analysis

One hundred and twenty-eight individuals of *T. clareae* and sixteen individuals of *T. koenigerum* (N = 8 from each colony, Tab. I) were genetically analyzed by three selected RAPD primers (OPA07, GAAACGGGTG; OPA11, CAATCGCCGT and OPA12, TCGGCGATAG) using conditions described by Klinbunga et al. (2000).

The amplification reaction was carried out for 40 cycles using a Perkin-Elmer Cetus DNA thermocycler (model 2400) consisting of denaturation at 92 °C for 30 s, annealing at 36 °C for 45 s and extension at 72 °C for 2 min. The final extension was performed at 72 °C for 5 min (Okurama et al., 1993). RAPD products were electrophoretically analyzed through 1.5% agarose gels and visualized using a UV transilluminator after ethidium bromide staining (Maniatis et al., 1982).

2.6. Data analysis

ITS sequences were aligned using Clustal W (Thompson et al., 1994). Sequence divergence was calculated according to the two-parameter method (Kimura, 1980) using Dnadist routine in PHYLIP (Felsenstein, 1993).

Each RAPD fragment was treated as an independent character and recorded in a binary matrix to represent an absence (0) or presence (1) of a particular band. The similarity index among individuals within samples was calculated by $S_{xy} = 2n_{xy}/(n_x + n_y)$, where n_x and n_y represent the number of RAPD bands in individuals x and y, respectively

Table I. Sampling locations and numbers of *T. clareae* and *T. koenigerum* individuals used for RAPD and sequencing analyses. One colony was sampled per location. The location number corresponds to the number in Figure 1.

Bee mite	Host	Location number	Sample abbreviation	No. of specimens	
				RAPD analysis	ITS sequencing
T. clareae	*A. dorsata*	2 (Lumpang)	TC2D	8	2
		7 (Nakhon Ratchasima)	TC7D	8	1
		9 (Samut Sakorn)	TC9D	8	3
		10 (Chanthaburi)	TC10D	8	0
		11 (Trat)	TC11D	8	2
		12 (Prachuap Khiri Khan)	TC12D	8	1
		13 (Chumporn)	TC13D	8	1
		14 (Samut Songkram)	TC14D	8	0
	A. mellifera	1 (Chaing Mai)	TC1M	8	1
		3 (Uttaradit)	TC3M	8	2
		4 (Udon Thani)	TC4M	8	1
		5 (Phisanulok)	TC5M	8	1
		6 (Khon Kaen)	TC6M	8	1
		8 (Bangkok)	TC8M	8	1
		10 (Chanthaburi)	TC10M	8	2
		13 (Chumporn)	TC13M	8	1
			Total number (*N*)	128	20
T. koenigerum	*A. dorsata*	9 (Samut Sakorn)	TK9D	8	3
		10 (Chanthaburi)	TK10D	8	2
			Total number (*N*)	16	5

and n_{xy} represents the number of shared bands between individuals (Nei and Li, 1979). Between sample similarity ($\bar{S}_{ij}$) was calculated as the average of all possible comparisons of individuals across samples *i* and *j*. Genetic distances between pairs of geographic samples ($\bar{D}_{ij}$) were converted using the equation; $\bar{D}_{ij} = 1 - \bar{S}_{ij}$, (Lynch, 1990). A UPGMA phenogram among geographic samples of bee mites was constructed using Neighbor implemented in PHYLIP version 3.56c (Felsenstein, 1993).

3. RESULTS

Amplification of the entire ITS of *T. clareae* originating from different geographic locations and host species; *A. dorsata* ($N = 10$) or *A. mellifera* ($N = 10$) and *T. koenigerum* from *A. dorsata* ($N = 5$) yielded a 600 bp product. Length polymorphism of ITS was not observed.

ITS1 and ITS2 of both *Tropilaelaps* species were 243 bp and 71 bp (*T. clareae*) or 72 bp (*T. koenigerum*) in length flanked with 181 bp of 5.8S DNA (Fig. 2). The boundaries of the regions were estimated by homology with ITS sequence of Phytoseiide mites, *Neoseiulus fallaci* and *Trohlodrommus pyri* (Navajas et al., 1999). Intraspecific polymorphism was not found in either species. Divergence between *T. clareae* and *T. koenigerum* ITS was 3.82%.

ITS1 of *T. clareae* and *T. koenigerum* showed greater sequence divergence (8.9%) than did ITS2 (4.6%). Totals of 12 substitutions (5 transversions and 7 transitions) and 10 insertions/deletions were observed in ITS1.

```
                 18S      ITS1                                                      60
T. clareae       CGTAGTGAAC CTGCGGAAGG ATCATTACTG TCGCAAAGTC CATTCACTCC GTCGGCGAGC
T. koenigerum    .......... .......... .......... ....G..... .......... ..........

                                                                                   120
                 GAGTGGTGCT CGAATGATGT TCTAACCCTC TC-----CGC GGAGGCGACG GGAGAGGCAT
                 .......... .........C .......... ..TTCTC... .......G.. ..........

                              inITS5                                               180
                 CTGTGCCCAG TATCGTATGT ATTCCATTCG TATTGCGATC TGACTTCGGC TGTGAAGTTA
                 .........C .......... ....G..... ...C.....A .A........ ..........

                                                                                   240
                 GGCGCGCGTC GCCGGTGCGT CCGGTTTGAC ATGCTTTTCC ATTTAACTCG TGCTATGGAG
                 .........- .......T.. .........- -......... C..C...... ......C...

                                   5.8S                                            300
                 AAAAGAAGAA CGCATCAGGA CTCAATATGG GGGATCACTT AGTCCTTAAA TCGATGAAAA
                 ...--..... .......A.. .......... .......... .......... ..........

                                                                                   360
                 ACATTGTAAT TTGTGGAAAT TGATGTGAGT TGTGAAATTT TGTGAGCATT GTGTTTTTGA
                 .....A.... .......... .......... .......... .......... ..........

                              InITS4                                               420
                 ATGAAAATTT CAGCATGGAT GCCTTGTGTC TATGCTACAC TTGTTTCAGT ATATAACTCG
                 .......... .......... .......... .......... .......... ..........

                                   ITS2                                            480
                 TAGTATATGT ACTTACTATT GCCGT-ACGC AATGGTATAA AATCTCCACG GTCACGAGAG
                 ..C....A.. ...G...... .....T.T.. .......... ......G... ..........

                                              28S        525
                 TGATGCTGCC TGCTCAAGTT GACGTGTATC TGAAATCAAG TGTGA
                 .......... .......... .......... .......... .....
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Figure 2. Nucleotide sequences of ITS of *T. clareae* (N = 20, accession No. AF544013, http://www.ncbi.nlm.nih.gov/Genbank/index.html) and *T. koenigerum* (N = 5, accession No. AF544014, http://www.ncbi.nlm.nih.gov/Genbank/index.html). Starting positions of ITS1, 5.8S, ITS2 and 28S are bold and underlined. Internal sequencing primers (inITS4 and ITS5) are underlined. The recognition sites of *Mse* I (TT/AA) and *Sau*3A I (/GATC) in *T. clareae* are bold-italicized.

A TTCTC sequence was fixed in *T. koenigerum* and was not found in *T. clareae*. Only single point mutations from transition, transversion and insertion/deletion were observed in the ITS2 region while five substitutions (2 transitions and 3 transversions) were found in 5.8S rDNA.

Sequences of ITS of *T. clareae* (N = 20) and *T. koenigerum* (N = 5) indicated the possibility to simplify species identification of these bee mites from DNA sequencing to RFLP analysis. Restriction analysis of ITS with *Mse* I (TT/AA) and *Sau*3A I (/GATC) across randomly chosen *T. clareae* (N = 30) and *T. koenigerum* (N = 10) revealed that the former possessed haplotypes A and A whereas the latter exhibited B and B, respectively (Tab. II and Fig. 3).

Eighty-six reproducible RAPD bands were amplified from OPA17 (34), OPA11 (26) and OPA12 (26). Of which, 16 and 20 RAPD fragments were specifically found in all individuals of *T. clareae* and *T. koenigerum*, respectively (Tab. II). Thirteen shared RAPD

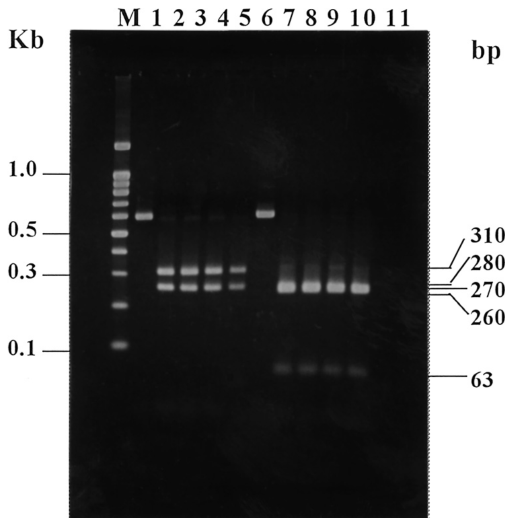

Figure 3. Patterns of undigested and *Mse* I-digested ITS of *T. clareae* (lanes 1 and 2-5) and *T. koenigerum* (lanes 6 and 8-10). A 100 bp DNA ladder was used as the DNA marker (lane M).

Table II. Species-specific markers of bee mites (Genera *Tropilaelaps*) in Thailand based on RAPD and RFLP analyses.

Species	DNA marker (bp)
T. clareae	OPA07; 1975, 1200, 850 and 640
	OPA11; 1650, 1150, 925, 540 and 430
	OPA12; 900, 720, 600 and 550
	ITS-*Mse* I; A (280, 260 and 63)
	ITS-*Sau*3A I; A (310, 150 and 140)
T. koenigerum	OP07; 2050, 2000, 1550, 1375, 1195, 1100, 610 and 430
	OPA11; 1500, 1450, 1300 and 810
	OPA12; 1650, 1425, 1140, 975, 895, 510, 480 and 385
	ITS-*Mse* I; B (330 and 270)
	ITS-*Sau*3A I; B (310 and 285)

fragments between these bee mites were observed. Ranges of similarity indices within colonies of *T. clareae* from *A. dorsata* (0.6943-0.9821) and *A. mellifera* (0.7736-0.9489) were comparable.

Genetic differences within species were much less than those between species (Tab. III). The average genetic distance within *T. clareae* from *A. dorsata* ($\overline{D}_{ij}$ = 0.1320) and *A. mellifera* ($\overline{D}_{ij}$ = 0.1008) was greater than that within *T. koenigerum* ($\overline{D}_{ij}$ = 0.0289). A UPGMA phenogram constructed from genetic distance between pairs of geographic samples showed clear differentiation between *T. clareae* and *T. koenigerum*. Within *T. clareae*, partial differentiation of bee mites from different host species was observed (Fig. 4).

4. DISCUSSION

DNA sequencing, PCR-RFLP and RAPD analyses have been used for population genetic and systematic studies in several taxa (White et al., 1996; Hall and Smith, 1991; Klinbunga et al., 2000; Sittipraneed et al., 2001). Using these approaches, several species-specific markers were observed in *T. clareae* and *T. koenigerum*. These markers can be used as species diagnostic markers to assist taxonomic difficulties of these bee mites in Thailand.

The entire ITS sequence of 20 individuals of *T. clareae* (519 bp) and 5 individuals of *T. koenigerum* (520 bp) did not reveal genetic heterogeneity within a given species suggesting that ITS polymorphism is not appropriate for genetic diversity studies of these bee mites at the intraspecific level. Sequence divergence between ITS of *T. clareae* and *T. koenigerum* was only 3.82% and reflected their genetically close relationship. In contrast, large genetic differences of ITS

Table III. Pairwise genetic distance among geographic samples of *T. clareae* and *T. koenigerum* calculated from RAPD analysis using primers OPA07, OPA11 and OPA12.

	TC2D	TC7D	TC9D	TC10D	TC11D	TC12D	TC13D	TC14D	TC1M	TC3M	TC4M	TC5M	TC6M	TC8M	TC10M	TC13M	TK9D	TK10D
TC2D	-																	
TC7D	0.1726	-																
TC9D	0.1467	0.0580	-															
TC10D	0.1345	0.0902	0.1066	-														
TC11D	0.0790	0.0857	0.0840	0.1213	-													
TC12D	0.1908	0.1410	0.1236	0.1197	0.1779	-												
TC13D	0.1461	0.0821	0.1134	0.0181	0.1214	0.1292	-											
TC14D	0.1598	0.1994	0.1843	0.1785	0.1763	0.1697	0.1847	-										
TC1M	0.1916	0.1739	0.1929	0.1962	0.1598	0.2010	0.1965	0.2013	-									
TC3M	0.1045	0.1773	0.1788	0.1667	0.1043	0.1861	0.1685	0.1471	0.1171	-								
TC4M	0.0659	0.1402	0.1462	0.1299	0.0605	0.1644	0.1432	0.1761	0.1280	0.0874	-							
TC5M	0.1843	0.1105	0.1191	0.1433	0.1353	0.1216	0.1520	0.2193	0.1295	0.1064	0.1070	-						
TC6M	0.0885	0.0929	0.0993	0.1229	0.0666	0.1638	0.1288	0.1883	0.1024	0.0774	0.0538	0.0917	-					
TC8M	0.1994	0.1287	0.1633	0.1401	0.1665	0.1467	0.1479	0.2314	0.1203	0.1143	0.1391	0.0571	0.1102	-				
TC10M	0.1402	0.1355	0.1133	0.1110	0.1323	0.1456	0.1144	0.1247	0.1446	0.0894	0.1376	0.1151	0.1022	0.1396	-			
TC13M	0.1290	0.0686	0.0829	0.1307	0.0814	0.1189	0.1416	0.1318	0.0840	0.0870	0.0673	0.0625	0.0637	0.1004	0.0859	-		
TK9D	0.8194	0.8693	0.8521	0.8421	0.8399	0.8700	0.8814	0.8510	0.8248	0.8052	0.8017	0.8450	0.8390	0.8576	0.8306	0.8356	-	
TK10D	0.8287	0.8786	0.8628	0.8524	0.8494	0.8814	0.8908	0.8613	0.8336	0.8121	0.8102	0.8552	0.8484	0.8685	0.8403	0.8463	0.0289	-

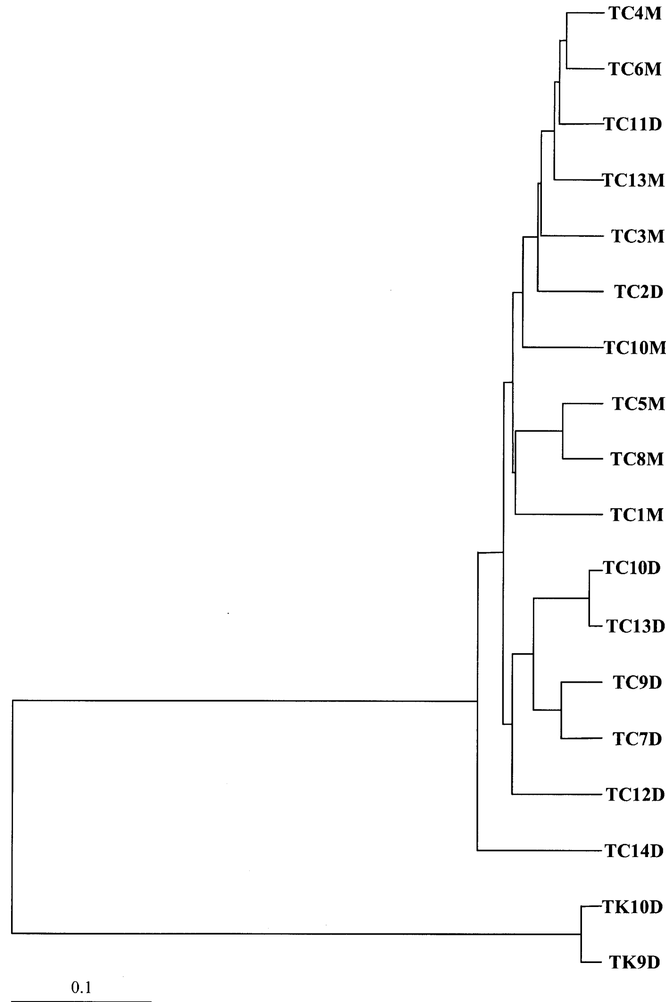

Figure 4. A UPGMA phenogram illustrating relationships of *T. clareae* and *T. koenigerum* originating from geographically different locations in Thailand based on RAPD analysis. A scale bar indicates genetic distance ($\bar{D}_{ij}$) of 0.1.

polymorphism (27.90–38.01%) were found between each of these species and *N. fallaci* and *T. pyri* (Navajas et al., 1999).

For general understanding of evolution and distribution of *T. clareae*, it is necessary to examine how often cross-species transmissions have occurred and the direction of transmission between different host species. The failure to detect intraspecific polymorphism in *A. mellifera*- and *A. dorsata*-originated *T. clareae* implies that additional genes having greater evolutionary rates than ITS should be used for such purposes, or that cross-species transmissions occurred recently.

Although a TTCTC stretch was consistently found in all individuals of *T. koenigerum* but not in *T. clareae*, this molecular marker cannot be directly applied for species diagnosis because it is cost-ineffective and time consuming when dealing with the large numbers of specimens. The existence of polymorphic *Mse* I and *Sau*3A I restriction sites in the ITS

of *T. clareae* and *T. koenigerum* allowed for simplification of species diagnosis by restriction analysis. However, this finding relied on limited individuals of *T. koenigerum*. Therefore, replicate sampling of non-Thai *T. koenigerum* over its geographic range is necessary before species diagnosis based on restriction analysis of ITS can be used unambiguously.

The major disadvantage for this study was sampling strategy. Basically, a large number of colonies representing accurate geographic origins of hosts are required. However, *A. mellifera* was an introduced species and has been exchanged between farms. Therefore the geographic origin of this species was obscure.

RAPD analysis using OPA07, OPA11 and OPA12 yielded 4, 5 and 7 *T. clareae*-specific fragments and 8, 4 and 8 *T. koenigerum*-specific fragments, respectively. Shared RAPD genotypes were not observed between these species. We did not find any RAPD fragment exhibiting host-specific nature in *T. clareae*. Therefore, apparent cross-species transmission and directions can not be concluded. The existence of *T. koenigerum* in Thailand has not been reported formerly. This is, therefore, the first report on a preliminary study on genetic diversity of *T. koenigerum*. A limited number of *T. koenigerum* was genetically examined in this study ($N = 16$). As a result, increasing the number of specimens would provide more accurate data about the level of genetic diversity in this species.

Although RAPD-PCR is sensitive to several reaction factors, it is quite useful when used with caution. To eliminate false negative results from RAPD analysis, sequence characterized amplified region (SCAR) markers can be further developed from RAPD markers for stable and accurate species diagnosis purpose (Klinbunga et al., 2000).

The UPGMA phenogram from RAPD analysis indicated large genetic differentiation between *T. clareae* and *T. koenigerum* and closer relationships of *T. clareae* from the same host than those between different hosts. A lack of phylogeography within *T. clareae* from different locations may have resulted from anthropological movement of *A. mellifera* over vast geographic areas. Recently, Insuan (2001) examined genetic diversity and population structure of *A. dorsata* origination from various locations in Thailand ($N = 154$) using restriction analysis of COI-COII, CytbI-tRNA^{ser}, ATPase 6-8 and lrRNA gene and microsatellites. Results revealed a lack of genetic heterogeneity of the mainland populations of this species. The potential ability of *A. dorsata* to migrate over long geographic distances reflects the high gene flow level in this species. This may have homogenized genetic differentiation, if any, of *T. clareae*.

In the present study, we demonstrate the successful development of species-diagnostic markers of two morphologically similar bee mites (*T. clareae* and *T. koenigerum*) in Thailand. Simplification of the detection method from DNA sequencing to restriction analysis of ITS allows practical implementation of DNA markers to resolve taxonomic difficulties of these bee mites in Thailand. Moreover, species-specific markers found in this study are useful for tracing the colonization of new hosts by these bee mites.

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Résumé – Différences génétiques entre *Tropilaelaps clareae* et *Tropilaelaps koenigerum* en Thaïlande sur la base des analyses par ITS et RAPD. Les acariens parasites d'abeilles, *Tropilaelaps clareae* et *T. koenigerum*, sont associés naturellement à *Apis dorsata* et *A. laboriosa*. *T. koenigerum* a été signalé la première fois en Thaïlande et *T. clareae* est maintenant signalé sur les cinq espèces du genre *Apis*. On a mis au point des marqueurs ITS (internal transcribed spacers), amplifiés par PCR (amplification génique), spécifiques aux deux acariens parasites en Thaïlande (Fig. 1 ; Tab. I) ainsi qu'une analyse par RAPD-PCR comme aides à la taxonomie de ces espèces. L'ITS a été amplifié à l'aide des amorces d'ITS5 et ITS4. Le produit de PCR (600 bp) a été analysé par électrophorèse et élué sur gel. L'ADN élué a été directement séquencé dans les deux sens à l'aide des ITS4, ITS5, inITS5 ou inITS4 comme amorces de séquençage.
L'ITS entier de *T. clareae* provenant de diverses localités géographiques et des deux espèces hôtes,

A. dorsata (N = 10) et *A. mellifera* (N = 10) et celui de *T. koenigerum* provenant d'*A. dorsata* (N = 5) ont montré que l'ITS1 et l'ITS2 des deux espèces de *Tropilaelaps* avaient une longueur de 243 paires de base (bp) et 71 bp pour *T. clareae* et de 72 bp pour *T. koenigerum* flanquées de 181 bp d'ADN 5.8S (Fig. 2). On n'a pas trouvé de divergence intraspécifique dans les séquences au sein de chaque espèce. La divergence entre les ITS de ces espèces selon la méthode à deux paramètres de Kimura était de 3,82 %. Une séquence fixe TTCTC a été trouvée chez *T. koenigerum* mais pas chez *T. clareae*. Les séquences nucléotidiques des ITS de *T. clareae* et *T. koenigerum* ont montré la présence de sites de restriction *Mse* I et *Sau*3A I polymorphes. L'identification de *T. clareae* et *T. koenigerum* s'est résumée à l'analyse de restriction de l'ITS amplifié avec *Mse* I et/ou *Sau*3A I (Fig. 3).
L'analyse génétique de 128 individus de *T. clareae* et de 16 individus de *T. koenigerum* a été faite à l'aide de trois amorces de RAPD sélectionnées (OPA07, OPA11 et OPA12). Elle a montré la présence respective 16 et 20 fragments spécifiques chez *T. clareae* et *T. koenigerum* (Tab. II). L'indice de similarité au sein des échantillons et entre les échantillons et la distance génétique entre paires d'échantillons géographiques ont été calculés. Les variations des indices de similarité au sein des populations de *T. clareae* sont comparables, que les acariens proviennent de colonies d'*A. dorsata* (0,6943-0,9821) ou d'*A. mellifera* (0,7736-0,9489). Un phénogramme UPGMA basé sur la distance génétique entre paires d'échantillons géographiques a clairement montré une nette différenciation entre *T. clareae* et *T. koenigerum* (Fig. 4). Une différenciation génétique partielle de *T. clareae* en fonction de l'hôte a été observée.

***Tropilaelaps* / acarien / marqueur diagnostique spécifique / diversité génétique / RAPD / ITS**

Zusammenfassung – Durch ITS und RAPD Analysen ermittelte genetische Unterschiede zwischen *Tropilaelaps clareae* und *Tropilaelaps koenigerum* in Thailand. Die parasitischen Bienenmilben *Tropilaelaps clareae* und *T. koenigerum* kommen natürlicherweise bei *Apis dorsata* und *A. laboriosa* vor. Zunächst wurde *T. koenigerum* in Thailand gefunden und nun wurde auch *T. clareae* bei fünf Honigbienenarten nachgewiesen. Spezifische diagnostische Marker der Bienenmilben *Tropilaelaps clareae* und *T. koenigerum* in Thailand (Abb. 1, Tab. I) wurden entwickelt, die auf der Sequenzierung von PCR-amplifizierten ITS (internal transcribed spacers) und RAPD-PCR beruhen, und die bei den Schwierigkeiten der taxonomischen Unterscheidung der Arten helfen sollen. ITS wurde mit den Primern ITS5 und ITS4 vermehrt. Das PCR Produkt (600 bp) wurde durch Elektrophorese analysiert und aus dem Gel eluiert. Die eluierte DNA wurde direkt in beide Richtungen mit ITS4, ITS5, inITS5 oder inITS4 als Sequenzprimer sequenziert. Die gesamten ITS von *T. clareae* aus verschiedenen geographischen Gegenden und von den Wirtsarten *A. dorsata* (*N* = 10) und *A. mellifera* (*N* = 10) sowie von *T. koenigerum* von *A. dorsata* (*N* = 5) ergaben Basenlängen für ITS1 und ITS2 bei beiden *Tropilaelaps* Arten von 243 bp und 71 bp (*T. clareae*) bzw. 72 bp (*T. koenigerum*) flankiert von 181 bp der 5.8S DNA (Abb. 2). Intraspezifische Divergenz in der Sequenz der Arten wurde nicht gefunden. Die Divergenz zwischen ITS dieser Arten beträgt nach Kimuras zwei-Parameter Methode 3,82 %. Eine festgelegte TTCTC Sequenz wurde bei *T. koenigerum* gefunden, aber nicht bei *T. clareae*. Nucleotidsequenzen der ITS von *T. clareae* und *T. koenigerum* ergaben polymorphe *Mse* I und *Sau*3A I Restriktions-Orte. Daraus ergibt sich, dass die Identifikation von *T. clareae* und *T. koenigerum* vereinfacht wurde auf eine Restriktionsanalyse von amplifiziertem ITS mit *Mse* I und/oder *Sau*3A I (Abb. 3).
Von *T. clareae* wurden 128 Einzeltiere und von *T. koenigerum* wurden 16 Einzeltiere genetisch mit 3 selektierten RAPD Primern (OPA07; OPA11 und OPA12) analysiert. Die RAPD Analyse ergab 16 bzw. 20 artspezifische Fragmente bei *T. clareae* und bei *T. koenigerum*, (Tab. II). Der Index für Ähnlichkeiten innerhalb und zwischen den Proben und genetische Distanzen zwischen Paarungen von geographischen Proben wurde berechnet. Der Bereich der Ähnlichkeitsindices innerhalb von *A. dorsata* Völkern für *T. clareae* (0,6943-0,9821) und in *A. mellifera* Völkern (0,7736-0,9489) waren vergleichbar (Tab. III). Ein UPGMA Phenogram, das auf genetischen Distanzen zwischen den gepaarten geographischen Proben basiert, zeigte eine deutliche Differenzierung zwischen *T. clareae* und *T. koenigerum* (Abb. 4). Eine partielle genetische Differenzierung von *T. clareae* in unterschiedlichen Wirtsarten wurde beobachtet.

***Tropilaelaps* / Bienenparasit/ RAPD / ITS / spezifische diagnostische Marker / genetische Diversität**

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